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Research paper

The iron component of particulate matter is antiapoptotic: A clue to the development of lung cancer after exposure to atmospheric pollutants?

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ABSTRACT

The classification of outdoor air pollution as carcinogenic for humans strengthens the increasing concern about particulate matter (PM). We previously demonstrated that PM exposure produces an antiapoptotic effect resulting from polycyclic aromatic hydrocarbons (PAH) and water-soluble components. In this study, we investigated transition metallic compounds, particularly iron, in order to decipher their underlying molecular mechanisms that prevent apoptosis.

Human bronchial epithelial cells were exposed for 4 h to different PM samples with established antiapoptotic effect (e.g. PM-AW) or not (e.g. PM-VS) or to their metallic components (Fe, Mn, Zn and Al) before apoptosis induction by the calcium ionophore A23187 or Staurosporine. PM-AW, Fe, Mn and Al significantly reduced induced apoptosis. The antiapoptotic effect of Fe was enhanced by benzo(a)pyrene, a typical PAH compound, but was totally reversed by the iron chelator, deferiprone. Furthermore, particles and iron triggered cellular ROS generation and prevented the depletion in glutathione levels observed during A23187-induced apoptosis. In contrast to benzo(a)pyrene, PM-AW and Fe rapidly activated NRF2, subsequently upregulated several target genes (*HO1*, *NQO1* and *GPX1*) and modulated some genes which control cell death (*BCL2*, *BAX* and *p53*). The key role of the NRF2 pathway in the antiapoptotic effect mediated by Fe and PM was demonstrated using siRNA technology.

Our results suggest that the iron component participates in the antiapoptotic effect of PM by activating a NRF2-dependent antioxidant process. As resisting to cell death is one of the hallmarks of cancer cells, these findings provide additional clues for understanding the development of lung cancer after atmospheric pollution exposure.

1. Introduction

Air pollution, especially particulate matter (PM), generates health concerns (particularly in urban areas with high population densities and industrial activities) because of the increased risk of cardiorespiratory morbidity and mortality [1], lung cancers [2] and the reduction in life expectancy [3]. These deleterious effects are related mainly to the smallest particles. They are able to reach the bronchi and alveoli and to accumulate in tissue and lung cells [4] which leads to respiratory and systemic inflammation through increased reactive oxygen species (ROS) production [5,6]. These airborne particles, termed PM_{10}, $PM_{2.5}$, and $PM_{0.1}$, have aerodynamic diameters equal to or less than 10, 2.5, and 0.1 microns, respectively. More than one thousand epidemiological studies have shown a significant association between PM exposure and a risk of lung cancer resulting in the classification of outdoor air pollution and PM as human carcinogens (Group 1) [7].

Carcinogenesis is a multistep process that includes initiation, promotion and tumor progression. It results in the development of a primary tumor and, eventually, the spread of the cancer to secondary sites through metastasis. The hallmarks of cancer consist of ten biological capabilities acquired during this multistep development and includes resistance to apoptotic cell death [8,9].

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Abbreviations

A23187	calcium ionophore (calcimycin)
AhR	aryl hydrocarbon receptor
ARE	antioxidant response elements
BaP	benzo(a)pyrene
BaP-AW	BaP *quantum satis* 10 μg/cm^2 of PM-AW
B2M	beta-2-microglobulin
DFP	deferiprone
D-man	D-mannitol
fE	control filter extract
Fe-AW	Fe *quantum satis* 10 μg/cm^2 of PM-AW
Fe-VS	Fe *quantum satis* 10 μg/cm^2 of PM-VS
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GPX1	glutathione peroxidase 1
GSTP1	glutathione S-transferase pi 1
HO1	heme oxygenase-1
MMP	mitochondrial membrane permeabilization
NQO1	NAD(P)H–quinone oxidoreductase 1
NRF2	Nuclear factor E2-related factor 2
PAH	polycyclic aromatic hydrocarbons
PM	particulate matter
PM-AW	$PM_{2.5}$ from Porte d'Auteuil collected in winter 2003
PM-VS	$PM_{2.5}$ from Vitry-sur-Seine collected in summer 2003
ROS	reactive oxygen species
SOD2	superoxide dismutase 2
XRE	xenobiotic response elements
ΔΨm	mitochondrial transmembrane potential

Apoptosis is defined by specific morphological alterations [10] as well as the externalization of phosphatidylserine (PS) and the permeabilization of plasma and organelle membranes [11]. These latter features arise from the activation of caspases which can be elicited by two distinct mechanisms: an extrinsic pathway, which involves transmembrane death receptors, or an intrinsic pathway which converges on the permeabilization of mitochondrial membranes (MMP). MMP is the checkpoint of the apoptotic process characterized by a decrease in the mitochondrial transmembrane potential (ΔΨm), the production of ROS, and the release of cytochrome c [12]. Importantly, these phenomena are affected by exposure to PM. Studies performed *in vivo* in rodents or *in vitro* on human bronchial epithelial cell lines following exposure to PM or their components showed an induction of apoptosis that was characterized by oxidative stress, decreased ΔΨm, caspase-9 activation, and DNA fragmentation [13–19].

The effects on apoptosis have been attributed to particular components of PM. The urban aerosol contains $PM_{2.5}$ and $PM_{0.1}$, which consist of a core of elemental carbon that adsorbs some inorganic components (ammonium, chloride, sulfates, nitrates, and metals) and organic compounds such as alkanes, alkanoic acids, aliphatic acids, quinones, polycyclic aromatic hydrocarbons (PAH), and biological species [6,20]. PM-induced apoptosis often has been attributed to various organic components, such as PAH, which activate the aryl hydrocarbon receptor (AhR) [19–21]. Heavy and transition metals adsorbed on PM also promote apoptosis *via* ROS generation, mitochondrial dysfunction, activation of caspases, or downregulation of antiapoptotic BCL2 proteins [22,23].

Most of the experiments which have shown PM-induced apoptosis were carried out with high doses of particles [13,21]. In contrast, we have performed *in vitro* exposure to $PM_{2.5}$ at low to moderate concentrations (i.e. from 1 to 50 μg/cm^2, [24]) that could mimic realistic exposure considering that $PM_{2.5}$ deposition in the tracheobronchial region is 2.3 μg/cm^2/24 h according to the modeling data of Li et al. [25]. We previously showed that exposure to PM samples collected from Paris prevents apoptosis in human bronchial epithelial cells that were treated with three broad-spectrum apoptosis inducers (A23187, staurosporine, and oligomycin). We found that the antiapoptotic effect of PM is related to their chemical composition. The exposure to certain particulate samples (of which PM-AW, the $PM_{2.5}$ from Porte d'Auteuil collected in winter 2003) inhibits apoptosis whereas the exposure to one chemically-distinct batch (PM-VS, the $PM_{2.5}$ from Vitry-sur-Seine collected in summer 2003) does not. We further found that the antiapoptotic effect of PM-AW is linked to PAH, particularly benzo(a)pyrene (BaP), *via* activation of AhR [24].

PAH and metallic compounds are notorious effectors of cellular exposure to PM by provoking an oxidative stress and activation of the ROS-sensitive transcription factor NRF2 (or NFE2L2, Nuclear factor E2-related factor 2) [26,27]. Under basal conditions, NRF2 is sequestered in the cytoplasm due to its interaction with two KEAP1 (Kelch-like ECH-associated protein 1) molecules, which facilitate its ubiquitination and proteasomal degradation. Under oxidative conditions, inducers alter KEAP1 cysteine residues which prevent its interaction with NRF2 [28]. NRF2 then becomes phosphorylated, translocates to the nucleus and binds to antioxidant response elements (ARE) localized in the promoter region of genes which code for antioxidant, phase II detoxifying enzymes and cytoprotective proteins such as heme oxygenase-1 (HO1), NAD(P)H-quinone oxidoreductase 1 (NQO1), glutathione S-transferases (GST), γ-glutamyl cysteinyl synthetase, glutathione peroxidases (GPX), and itself [29].

Since our previous work demonstrated that exposure to $PM_{2.5}$ triggers an antiapoptotic effect which is due to the water-soluble and PAH components adsorbed on particles, the aim of the present study was to further elucidate the molecular mechanisms involved in this cytoprotective effect, with a particular focus on the transition metals of PM, ROS production, NRF2 activation and glutathione homeostasis. We found that the antiapoptotic effect caused by BaP takes place independently of the NRF2 pathway whereas iron, a typical transition metal of PM, activate the NRF2 transcription factor and upregulate the expression of its target genes thus allowing the maintenance of GSH levels.

2. Materials and methods

2.1. Particles collection and preparation

Urban $PM_{2.5}$ were collected in Paris during the summer or winter of 2003 at two locations: an urban background station in the Paris suburb of Vitry-sur-Seine (PM-VS) and a curbside traffic station at the Porte d'Auteuil which borders the ring road of Paris (PM-AW) [24]. The chemical and morphological characterization of PM used here has been reported previously [30]. All particles were suspended at 2 mg/mL in supplemented DMEM/F12 medium (Gibco®) and stored at −20 °C. PAH-naked particles were prepared by extracting the organic compounds with dichloromethane (Sigma–Aldrich®) and the particle pellets were re-suspended in DMEM/F12 medium. As an additional check of the method, a control filter extract (fE) was produced in the same way from unloaded nitrocellulose filters.

The chemical and morphological characterization of PM used herein has been previously reported [30]. In all batches, soots are the most important particles generally consisting, at their emission